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From: STIC-Biotech/ChemLib
Sent: Thursday, January 23, 2003 6:12 AM
To: STIC-ILL
Subject: FW: Journal Article Needed

-----Original Message-----

From: Young, Josephine
Sent: Wednesday, January 22, 2003 8:21 PM
To: STIC-Biotech/ChemLib
Subject: Journal Article Needed

I would like to request the following article/abstract for case no. 09/700,751:

Fishman, P. et al. "Adenosine acts as a chemoprotective agent: A new mechanism" Proceedings of the American Association for Cancer Research Annual Meeting (March 1999), Vol. 40, p. 677 (Meeting info: 90th Annual meeting of the American Association for Cancer Research, Philadelphia, PA, April 10-14, 1999 American Association for Cancer Research).

AN (from STN) 1999:191711 BIOSIS

Thanks for all your help!

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#4465 Ethnic variation in the thymidylate synthase enhancer region polymorphism. Sharon Marsh, Margaret M. Ameyaw, Elaina S.R. Collie-Duguid, Tao Li, Jessie Githang'a, Anne Indalo and Howard L. McLeod. *Oncology Laboratory, Department of Medicine and Therapeutics, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, Scotland; Centre for Tropical Clinical Pharmacology, University of Ghana Medical School Accra, Ghana; West China University of Medical Sciences, Chengdu, Sichuan 610041, PR China and Kenyatta National Hospital, University of Nairobi, Kenya.*

Thymidylate Synthase (TS) catalyses the methylation of dUMP to dTMP, providing the only *de novo* source of thymidylate in the cell. This makes it a valuable target for chemotherapeutic agents such as 5-fluorouracil and raltitrexed. Over-expression of TS has been linked to resistance to these drugs. One mechanism of over-expression may come from a polymorphism of a 28bp tandem repeat in the enhancer region of the TS gene. *In vitro* studies suggest that the presence of a triple repeat can give 2.6 times higher TS gene expression than a double repeat. Analysis of this region in 96 Caucasian, 95 Southwest Asian, 96 Chinese, 92 African American, 166 Ghanaian and 98 Kenyan subjects showed significant differences in the allele frequencies and genotype distribution for the double and triple repeats. The percentage homozygous for the triple repeat in Chinese was 66%, Southwest Asians 41%, Ghanaians 30%, Caucasians 28%, Kenyans 26% and African Americans 22%. Novel alleles were also found in Kenyan, African American and Ghanaian populations. Sequence analysis showed these to contain 4 and 9 copies of the tandem repeat. If increasing the tandem repeat region leads to greater expression of TS, ethnic variation in this region may result in significant differences in the effectiveness of TS targeted chemotherapy, making some populations more resistant to routinely used TS inhibitors. Direct analysis should now be used to assess the impact of these ethnic variations on the use of TS targeted chemotherapy agents.

#4466 Modulation of dihydropyrimidine dehydrogenase activity by parenteral nutrition in tumor bearing rats. Tsujinaka, T., Taniguchi, M., Yano, M., Kaneko, K., Akiyama, Y., Miki, H., Monden, M. *Department of Surgery II, Osaka University Medical School, Osaka 565-0821, Japan.*

Dihydropyrimidine dehydrogenase (DPD) is a rate-limiting enzyme for 5-fluorouracil (FU) catabolism, the activity of which may be modulated by a nutritional state. Yoshida sarcoma cells were implanted to the backs of male Donryu rats. After 7 days, tumor formation was confirmed and the rats were divided into two groups: FED group (n = 18) received non-purified rat chow and infusion of saline via a PN route; PN group (n = 16) received a standard PN regimen via a PN route. On day 6, half rats of each group received infusion of FU for 24 h (40 mg/kg/d). On day 7, rats were sacrificed at 10 AM to measure DPD activities of the liver and the tumor, FU concentrations of the serum and the tumor, and thymidylate synthase (TS) levels in the tumor.

	h-DPD act.	s-FU conc.	t-FU conc.	total TS	free TS	bound TS
FED	1.12±0.14	100.3±18.4	228.3±35.1	39.9±8.5	26.5±5.5	13.4±3.5
PN	0.93±0.12	143.1±19.5	377.2±118.8	48.0±2.8	31.1±1.8	16.9±1.4
P value	0.0045	0.0003	0.0435	0.0242	0.0444	0.0189

PN induced a decrease of hepatic DPD activity and an increase of serum and tumor FU concentrations. Total, free and bound TS levels were increased on PN. The activity of DPD of the tumor was below a detection level. The results showed that PN suppresses the activity of hepatic DPD and inhibits the catabolism of FU. The action of FU may be increased in a patient on PN.

#4467 Transcription initiation by RNA polymerase II is abated by 2-chlorodeoxyadenosine insertion into DNA. Hartman, W.R. and Hentosh, P. *Finch University of Health Sciences/The Chicago Medical School, North Chicago, IL 60064.*

The nucleoside analog, 2-chlorodeoxyadenosine (CldAdo, 2-CdA), has proven effective in the treatment of hairy cell leukemia and chronic lymphocytic leukemia. Previously we demonstrated by computer-generated models and *in vitro* gel shift assays that binding interactions of the human transcription factor TFIID were disrupted on CldAMP-substituted TATA box consensus sequences. We hypothesized that human RNA polymerase II (pol II) transcriptional processes would therefore be affected by CldATP incorporation into DNA. Double-stranded DNA templates containing the adenovirus major late promoter and coding sequences were enzymatically synthesized as a control or with a site-specific CldAMP residue(s), incubated with HeLa extract, and synthesis of full-length, radiolabeled 44-base transcripts was assessed. When CldAMP was substituted for dAMP within the TATA box, *in vitro* pol II transcription decreased by 33.5% (+/- 9.6) compared to control substrates. Time course studies showed that transcript production increased in a linear fashion over a 60 min period in control reactions. In contrast, transcription on CldAMP-substituted TATA sequences reached a plateau after 20 min. This finding indicates that multiple rounds of transcription could not be initiated, possibly due to sequestering of transcription factors and/or RNA pol II by the substituted promoter sequences. Diminished transcription due to CldATP insertion in eukaryotic promoters, as well as interference with RNA pol

II elongation when inserted in coding regions may partially account for cytotoxicity associated with CldAdo treatment. Supported by Grant CA55414 and Sigma Xi, GIAR.

#4468 Adenosine acts as a chemoprotective agent: A new mechanism. Fishman, P., Bar-Yehuda, S., Farbstein, T., Bahaar, F. *Felsentein Medical Research Center, Sackler Faculty of Medicine, Tel Aviv University, Rabin Medical Center, Petah Tikva, Israel 49100.*

Adenosine, a purine nucleoside, exerts its activity via interaction with G-protein associated A1 and A2 cell surface receptors. In most cells, A1 adenosine receptors are coupled to the inhibition of adenylate cyclase, while A2 receptors mediate activation of the enzyme. We have recently shown (Cancer Research 58, 3181-3187, 1998) that adenosine exhibits a differential effect on tumor and normal cell growth. Whereas proliferation of tumor cells was inhibited, normal cells such as bone marrow, were stimulated following treatment with adenosine. Adenosine's capability to induce bone marrow cell growth, has prompted us to test its ability *in vivo* to prevent myelotoxicity caused by chemotherapy. Indeed, adenosine, given prior to chemotherapy, increased the peripheral number of leukocytes and neutrophils (which were reduced following treatment) and induced an *ex vivo* bone marrow cell proliferation. This study focuses on the role of G-CSF and the A1 adenosine receptor in mediating the chemoprotective effect of adenosine. Adenosine was found to stimulate G-CSF production by peripheral blood human mononuclear cells or by murine bone marrow cells. DMPX, a selective adenosine A1 receptor antagonist, neutralized the stimulatory effect of adenosine as well as the G-CSF production. DPCPX, an A2 receptor antagonist, failed to negate both activities. We concluded, therefore, that the stimulatory effect of adenosine on bone marrow cells, can be attributed to the A1 adenosine receptor. Since patients are currently being treated with adenosine (e.g. in cases of arrhythmia), its application as a chemoprotector may be facilitated.

#4469 Down regulation of I¹²⁵-EGF binding and epidermal growth factor receptor (EGFR) expression in drug resistant neuroblastoma cells. Mirkin, B.L., Clark, S., and Tham, E. *Northwestern Univ. Med. School, Children's Mem. Inst. for Edu. and Research (CMIER), Chicago, IL 60614.*

The replication rate of drug resistant neuroblastoma (R-NB) cells is slower than that of wild, non-resistant (W-NB) cells. A putative mechanism for this may be that R-NB cells are less responsive to mitogenic growth factors, such as EGF. We have previously demonstrated that I¹²⁵-EGF binding can be down regulated by prolonged incubation of W-NB with NGF or by transfection with the NGF gene (Proc. AACR, 37:41, 1996). This study has compared I¹²⁵-EGF ligand binding and total EGFR expression in W-NB and R-NB cells. A competitive radioligand binding assay was used to quantitatively determine specific I¹²⁵-EGF binding to intact cells. EGFR expression was analyzed by Western blot utilizing a polyclonal EGFR antibody and densitometric scanning. C-1300 murine and SK-N-SH human neuroblastoma (NB) cells made resistant to the nucleoside analogue (Z)-5'-fluoro-4', didehydro-5' deoxyadenosine (MDL) or doxorubicin were compared with W-NB parent cells, as controls. The binding of I¹²⁵-EGF to NB cells resistant to MDL was 32.9 ± 6.7% (N=5) of W-NB cells; doxorubicin resistant NB cells bound approximately 50% (N=1) of W-NB controls. Densitometric analysis of Western blots revealed an 85% decrease in the EGFR expression of R-NB cells when compared to W-NB. Conclusion: The binding of I¹²⁵-EGF to EGFR, expression of EGFR and mitogenic response to EGF are significantly reduced in R-NB cells, resistant to either MDL or doxorubicin.

#4470 Characteristics of 2-chloro-2'-deoxyadenosine (CdA) and fludarabine (Fara-A) acquired resistance in a human promyelocytic cell line. Månsson, Emma., Spsokoukotskaja, Tanja., Jönsson, Sofia., Eriksson, Staffan., Albertoni, Freidoun. *Department of Clinical Pharmacology, Sweden. Medical Chemistry, Molecular Biology and Pathobiology, Budapest, Hungary and Department of Veterinary Medical Chemistry, The Biomedical Center, Uppsala, Sweden.*

CdA and Fara-A are nucleoside analogues with remarkable antineoplastic activity *in vitro* and *in vivo*. The mechanisms by which these analogues exert their cytotoxic effects are not clearly understood. Two stable resistant cell lines were developed from HL-60 cell line by exposure to increasing concentrations of CdA (HL-60/CdA) and Fara-A (HL-60/Fara-A) over a period of 8 months. HL-60/CdA and HL-60/Fara-A cells tolerate more than 8000 and 5-8 fold higher concentrations of CdA and Fara-A respectively. Neither the percentage of cells in G1 and S phase, measured before and after 4 drug-free passages, nor the doubling times differed significantly in any of the cell lines. The activity of the nucleoside phosphorylating enzyme, deoxycytidine kinase (dCK) in HL-60/CdA was about 5%, as compared to the levels in the parental cell line and this dCK deficiency is most likely the mechanism of resistance. The corresponding dCK level in HL-60/Fara-A cells was 60%. The mitochondrial enzyme, deoxyguanosine kinase was not affected in the resistant cells. The resistant cells showed considerable cross-resistance to cytarabine, difluorodeoxycytidine and difluorodeoxycytidine toxicity, most likely due to the decreased phosphorylation of these analogues by dCK. The cytotoxicities of tubercidine, daunorubicin, vincristine and paclitaxel showed similar toxicity profiles in resistant cells compared to the wild type. Since ribonucleotide reductase (RNR) is a primary target for CdA and Fara-A nucleoside